**Original Research Article**

# Prevalence of Malaria and a comparative study of peripheral blood smear, Quantitative buffy coat and malaria antigen rapid test methods in the diagnosis of malaria from Dakshina Kannada District, India

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**A B S T R A C T**

| **Keywords** | Malaria is a serious vector borne parasitic infection world wide in distribution. Dakshina Kannada district of Karnataka is endemic for malaria and poses a diagnostic challenge in the medical community. Patients attending Dr. M. V. Shetty Hospital, situated at the heart of the Mangalore city were chosen for the study. A total of 4383 patients were tested by QBC alone. Fifty eight samples of clinically suspected malaria were also tested by peripheral blood smear(PBS), Quantitative Buffy Coat(QBC), and Antigen Rapid Test methods and the results were compared. Out of a total of 4383 samples tested by QBC method, 1903(43.42%) were positive for malaria. A comparison of QBC with conventional thick blood and Malaria antigen rapid methods yeilded 96.55% ,72.41% and 94.82% positivity respectively. Mangalore city has a high rate of malaria infection. QBC, PBS and Antigen detection methods are equally efficient in diagnosing malaria. |
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| Malaria; Peripheral blood smear; QBC; Antigen detection. | |

## Introduction

Malaria caused by Plasmodium species, *P.vivax, P.malariae, P.falciparum* and *P.ovale* transmitted by female Anopheline mosquitoes poses a diagnostic challenge to medical community world wide. Over 90% of Malaria is caused by *P.vivax* and *P.falciparum.* Now malaria is mostly confined to tropical and subtropical areas of the world; the warm climate, heavy rain fall and stagnant water provide an ideal environment for mosquitoe breeding in these areas. Malarial paroxysm is the characteristics defining clinical symptoms of the disease including cold stage, hot stage and sweating stage and the other characteristic symptoms such as fever, chills, body pain, head ache, nausea, vomiting, diarrhoea etc., Malaria due to *P.falciparuum* is fatal due to multi system disease associated with complications such as cerebral malaria, algid malaria, septicemic malaria, severe anaemia, kidney failure, acute respiratory distress syndrome, metabolic acidosis, hypoglycemia etc (malaria@cdc.gov, 2010). Occurrence of malaria encomposes

90 countries with over 50 million cases and 1.1 to 2.2 million deaths every year was reported. In India in the year 2005 1.8 million cases were reported, of which 44.5% were caused by *P. falciparum* (Parija *et al*., 2009). The reported cases of malaria for Karnataka in 2010 were 44122 cases of which 7771(17.61%) were caused by *P. falciparum.* In Dakshina Kannada district, of the 7025 reported cases, 984(14.00%) were falciparum malaria (NVBDCP data, 2011). During 2005 a total of 20083 cases of malaria were reported from Mangalore city (Kakkilaya, Malaria site.com 2012) which is very high compared to the population density of the city.

Clinical diagnosis of malaria is extremely difficult even to an experienced medical practitioner. Reliable laboratory methods are needed to assist the clinical diagnosis of malaria. Considering this, a qualitative comparison of peripheral blood smear, QBC and antigen detection methods in the diagnosis of malaria was done in the present study.

## Materials and Methods

A total of 4383 blood samples were collected from patients with or without symptoms of malaria attending Dr. M. V. Shetty Hospital, Mangalore, during the period from January 2008 May 2010. The samples were processed for malaria by quantitative buffy coat method. Blood samples for malaria were cross checked by Leishmann stained peripheral blood thick smear for confirmation of the findings.

Another 58 blood samples collected from clinically suspected cases of malaria were tested by QBC, PBS and antigen detection by immunochromatographic method (SD Bioline).

## QBC

In the QBC system, the parasites were concentrated by centrifuging the blood samples in a special capillary tube quoted with acridine orange and an anticoagulant. The tube contained a small plastic cylinder (float) approximately 55 to 60 micro litre of blood was centrifuged at 1200 rpm per minute for 5 minutes. Following centrifugation the white blood cells, platelets and red blood cells infected with malarial parasites accumulated in the space between float and inside wall of the capillary tube was observed under a microscope fitted with paralens ultraviolet microscope adaptor and a X60 objective connected to fibre optics UV light module.

## PBS

Thick peripheral smears of blood samples prepared as per standard procedure were stained with the Leismann's stain and microscopically examined for malarial parasites under oil immersion objective. A total of 200 to 300 microscopic fields were examined before the film was declared negative.

## Malaria Antigen Rapid Test (SD bioline malaria antigen test)

It is a immuno diagnostic strip test. It contained a membrane strip pre coated with monoclonal antibody and polyclonal antibody as the separate lines across a test strip. The former was specific to *Plasmodium falciparum* HRP - II protein and the latter specific to lactate dehydrogenase of Plasmodium species. A drop of assay diluent and 4 drops of assay buffer were dispensed in to the conjugate and washing wells, respectively. One drop of freshly collected blood was added to the conjugate wells and mixed gently with a dropper. After standing for 1 minute,

placed the dipstick vertically in to the conjugate wells, which contained the specimens, and left it in the well for 10 minutes. Then transferred the dipstick vertically from the conjugate wells in to the washing wells and left it was cleared of blood and the procedure control band became clearly visible. Then removed the dipstick from the washing well and read the results (Seiwon Lee and Kyeongman Jeon, 2008).

## Result and Discussion

A total of 1903 (43.53%) were positive for malaria, out of the 4383 blood samples examined by QBC method. Out of a total of 58 samples of clinically suspected malaria tested by QBC, PBS and antigen detection methods, 56 (96.55%), 42 (72.41%) and 55 (94.82%) were positive for malaria respectively. (Table 1)

In early 1990s coastal Dakshina Kannada district of Karnataka state was relatively free from malaria with only sporadic case reports. But since 1990, with the sudden spurt in industrialization and construction activities, malaria made a dramatic comeback to this region (Kakkilaya, Malaria site.com, 2012).

In the year 2006, a total of 62864 cases of malaria were reported from Karnataka state and Mangalore accounted for 15664 (24%) of these cases. Of the 16446 cases of *P. falciparum* malaria reported from Karnataka in the same year, 4903 (29%) cases were from Mangalore and among 29 malaria related deaths from Karnataka, 11 (37.93%) were from Mangalore. There was a sudden rise in the cases of *P. falciparum* in the year 1995-96 that resulted in more than 100 deaths due to malaria (Kakkilaya, Malaria site.com, 2012).

Our study showed a total of 1903 (43.53%) were positive for malaria, out of the 4383 blood samples examined by QBC method. Out of a total of 58 samples of clinically suspected malaria tested by QBC, PBS and antigen detection methods, 56 (96.55%), 42 (72.41%) and 55 (94.82%) were positive for malaria respectively. Some study shown that the sensitivity of PBS is higher like our study (Bhandari *et al*., 2008). But some other studies shown low sensitivity for PBS (Fatima Shujatullah *et al*., 2006; Pinto *et al*., 2009).

In endemic area the diagnosis of malaria presents a methodological problem due to the non specific symptoms and high prevalence. Prompt and effective treatment is required to overcome the fatality. In this respect proper methodology for the diagnosis of malaria should be opted.

Characteristics of an ideal diagnostic tools for malaria are rapid availability of result, easy to use and minimal training requirements, minimal materials with long shelf life that do not require refrigerator or electricity, reproducible results, allows able to detect all malarial species including mixed infection, accurate enough to detect a low parasite density, able to quantify parasitaemia, revert to negative within a few days of clearing viable parasites and cost effective. No diagnostic tool needs all the above criteria due to the variations of the test depending on the epidemiology of infection, goals for control in the region where the test is used (Clinton. K Murray *et al.,* 2008).

The present study revealed a high occurrence of malaria in Dakshina Kannada district.

**Table.1** Comparison of PBS, QBC and Antigen detection SD bioline

| Diagnostic Test | Cases positive for malaria Study group = 58 |
| --- | --- |
| QBC | 56 (96.55%) |
| Blood smear examination | 42 (72.41%) |
| Antigen detection | 55 (94.82%) |

Early detection is very important to institute prompt treatment of the patients, in order to avoid complications. Although the conventional blood smear is highly specific and cheap, it has limitations such as need of a specialized microscopist. It may be used to cross check the positive results of other malaria diagnostic techniques. QBC even though provides fast diagnosis with high sensitivity, has disadvantages such as over diagnosis, less sensitivity than thick smear in detecting low parasitaemia (less than 100ml) and less sensitivity than parasight F.

Species differentiation is difficult and considerable skill and experience are required to process and examine the tubes correctly and confidently. Micro coagglutination, the adverse effects of heat and humidity, strong back ground fluorescence scattered by red blood cells, WBCs and incorrect positioning of float have been reported to be the difficulties associate with examining some tubes (Craigh and sharp, 1997; Lowe *et al*., 1996; Baird and Purnomo, Jones, 1992). Dip slide tests for the detection of plasmodial antigen tests are simple and rapid to perform, making it easy to teach the methodology to inexperienced or even untrained persons (Jelinek *et al*., 1993). However, although sensitivity was at high levels, having limitations like negative results cannot be ruled out malaria completely. Also it can be found in the blood several days after the parasites have cleared.

Since the prevalence of malaria is high in Mangalore city including falciparum malaria, the early detection is an important aspect. Therefore QBC may be recommended where facilities are available, where there is no availabilities of such facilities, simple and easy techniques like antigen detection can be used by cross checking the positive cases with PBS

**Acknowledgement**

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